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THE POSSIBLE MILITARY SIGNIFICANCE OF CONTAMINANTS FOUND IN TROPICAL ATMOSPHERES

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Introduction: I am from the Army's Tropic Test Center located in the Canal Zone. This Center, as some of you know, is one of the three installations which the Army has assigned to testing materials in a specific environment. The other two, at Yuma and Fort Greeley, exist for desert and arctic testing, respectively.

One of our tasks is to determine how materials react to deteriorative forces that are peculiar to the humid tropics. However, before too many of you ask--as many do--why we need more demonstrations of wood and fabric rotting and iron rusting, let me say, quickly, that we have an assignment to do more than repeat what has already been done.

What I will report today will be some of the work being done in microbiology and atmospheric chemistry. As background, however, let me say that our work involves observation of as many as possible of the elements of the tropical environment such as climate, vegetation, and soil, as well as microbiology and atmospheric chemistry. The object of the work is to learn more about the action of the elements of the environment on materials than has heretofore been possible. For example, it is common knowledge that microorganisms play a part in the destruction of organic materials. Annotated bibliographies on this subject, published through 1965, prepared at the Prevention of Deterioration Center (PDC)*, are voluminous. It has also been suspected for some time that microorganisms contribute to deterioration of metals. For the most part, reports on the possible role of microorganisms in metals deterioration deal with failure of

* Prevention of Deterioration Center (no longer active)
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metals in walls of tanks, submerged structures, or subterranean situations. Annotated bibliographies, PDC, 1965 and 1963, summarize most of the information on this subject, including the very few papers in existence which suggest that microorganisms may influence deterioration of metals exposed to ambient atmospheres.

In this report, I expect to describe some findings which seem to be related to practical problems, including a possible effect on metals exposed to the atmosphere. I also hope to illustrate the need to observe the tropical environment in terms of small increments of space and time. Throughout the paper, I will be pointing out the variations in the tropical environment that are dependent upon region, season, elevation above ground, and even degree of exposure to the general atmosphere.

Report of Results: Having stated that organic materials do suffer from attack by microorganisms and that metals may also be adversely affected, I'd like to begin with some special observations on iron. We all know, of course, that iron rusts. But there may be some incremental effects not heretofore observed that will extend what is known about this very familiar phenomenon.

In this experiment, very thin films of pure iron were exposed to the environment. These films, deposited on plastic surfaces by vacuum evaporation, are only about 1000 Angstrom units thick. They are so delicate that even the slightest change in their characteristics is readily noticeable. The technique of using thin metal films to observe deteriorative effects was devised at the National Center for Atmospheric Research (Lodge, 1962). In fact, the films you will be seeing were prepared by members of the staff of that organization. Films like this (Plate 1) mirror bright control plate were exposed under a variety of physical locations and the results were observed.

Unless another type of container or condition of exposure is specifically described as an experimental variable, all exposures were inside eighteen-inch lengths of five-inch diameter, fiberglass tubes screened at the ends with sixteen mesh nylon screen. These containers were used to protect the surfaces from direct sunlight and wind, as well as to prevent damage to the surface by insects and animals. When exposure was in an open area, tubes were placed perpendicularly to the prevailing wind.

Plate 2 shows two surfaces after seven days' exposure. These surfaces were, of course, identical to the control plate in the beginning. The rather fine grained texture of observed surface deterioration seen in a) is characteristic of all open to sky exposures between ground level and one hundred-fifty feet above the ground. The surface b) after (seven days') exposure under forest cover has developed roughly circular spots indicative of a different kind of attack, or at least an additional effect of the forest environment. These differences in texture can better be seen at thirty times magnification.

Plate 3 is Plate 2 a), the open to sky exposed surface, magnified. The texture of this surface is not really greatly different from a surface shown in Plate 4. Deterioration in Plate 4 was a controlled exposure to very fine sea water particles. The color difference between Plate 3 and Plate 4 is an effect of time. Plate 4 was photographed 48 hours after exposure. Since no location in the Canal Zone is very far distant from one of two oceans, and since salt particles are detectable in the air in most places, the result, primarily a salt effect, obtained from exposure in the open to sky condition was the expected one.

However, no such easy answer is available to explain why a different pattern of deterioration is seen in surfaces exposed below forest cover. The magnified pattern of this surface is shown in Plate 5. The concentric ring pattern visible on this surface suggests that whatever has happened may have started at a focal point and spread outward to form the irregular pattern of observed spots.

The concentric ring pattern appears under other kinds of exposure. For example, the two surfaces of Plate 6 were exposed, for seven days, inside closed but not gasketed shipping containers for Nike Ajax missiles. The altered surface a) came from a shipping container which had been under forest cover for eight months. This container had an accumulation of surface moisture and an abundance of fungal contamination on the interior walls. The fungus layer was heavy and could readily be seen and smelled. Plate 7 is the magnified view of the deteriorated surface shown in Plate 6 a). Note that the concentric ring pattern like that shown in Plate 5 is present. The unaltered surface b) was from an identical shipping container tube stored for more than a year in an open to sky cleared area. The interior of this container was dry and free from a visible layer of microorganisms on the walls of the interior surfaces. High daytime temperatures, due to direct exposure to sunlight, reasonably account for absence of both excess moisture and a high microbial population. Exposure to high daytime temperature does not, however, preclude the likelihood of nighttime cooling and the attainment of saturated atmospheres within the container leading to surface deposition of water films on interior surfaces at night.

On the basis of the surfaces discussed thus far, we might say that the exposure conditions which appear associated with the "concentric ring" type of film deterioration are high atmospheric moisture with frequent opportunities for change of temperature forcing saturation and surface wetting, the presence of microorganisms or their products, and the under forest cover environment. Please bear this in mind as the next few plates are shown.

In Plate 8, we see two other films quite different in appearance. Both of these were exposed for seven days under forest cover. We may say that a) is either from the eighteen-inch long tube used for protection of the exposure surface, or from the loosely closed missile

shipping container. Actually, it is the latter. It has been subjected to an environment high in microorganisms and moisture and has also been influenced by diurnal temperature change and effects of vertical air currents in such a way that frequent achievement of surface moisture deposition on interior surfaces is assured. On the other hand, surface b) was laid directly on the forest floor and was covered by a glass bell jar. A more complete exposure to moisture and microorganisms would be difficult to achieve; yet, note that the surface was unaffected. A possible reason why the surface placed directly on the ground was unaffected may be that, close to the ground, diurnal temperature changes are minimal and, in fact, almost nonexistent, and protection by the heavy glass bell jar from the cooling effect of night air and vertical air currents prevented frequent wetting conditions. At any rate, the type of attack of iron seen when all requisite conditions were present was not observed.

Considering all of the above exposure conditions and the observed results, we now have evidence that high moisture content of atmosphere associated with frequent temperature change and the presence of significant numbers of microorganisms and their products are requisites to the "concentric ring" type of iron deterioration. The "under forest cover" factor is not critical.

Continuing, let us consider that it was only when we subjected iron surfaces under bell jars to repeated wetting by condensing water vapor that we achieved anything like the "concentric ring" effect noted above. Plate 9 shows how chambers to obtain saturated atmospheres were made and used. In a) we see a saturated atmosphere chamber with the bulb at the top of the unit made cool to condense water needed for later atmospheric saturation. In b) the bulb has been warmed to evaporate the water and produce saturation inside the chamber. Plate 10 shows how iron surfaces placed on moistened litter on the forest floor, but kept inside the chamber and subjected to repeated condensation of water vapor, looked after seven days' exposure. The concentric ring appearance has developed to some extent. It is of interest to note here (not illustrated) that the deleterious effects noted were reduced if the (dry season) forest floor litter over which the surfaces were placed was not kept wet during the period of exposure.

Attempts to confirm, in the laboratory, the indication that conditions of high moisture, frequent temperature variation, and the presence of significant populations of microorganisms are needed to produce the concentric ring effect in iron deterioration were moderately successful. When saturated vapor units were set up over forest litter placed on wet sterile sand in the laboratory, we obtained a few typically mottled spots on the exposed film. These spots were characteristically concentric ringed as shown in Plate 11. On the other hand, parts of the exposed films in the laboratory exposures remained unaffected. This experiment gains credibility, however, when the fact is noted that no effect whatsoever was obtained, even after fifteen

days' exposure in parallel exposure trials in which the litter used has been cleared of living microorganisms by gaseous sterilization. Neither was any effect on exposed iron surfaces noted when they were exposed in a condensing chamber in the laboratory in which water was the only ingredient present.

To review, evidence is presented that thin films of pure iron exhibit a characteristic kind of "concentric ring" deterioration if conditions of high atmospheric content, frequent temperature cycling, and significant populations of certain microorganisms are present. In no instance was the characteristic "concentric ring" type of deterioration noted unless all three of the requisite conditions were present. This suggests, but certainly does not prove, that the metal iron may be influenced by microorganisms or products of microorganisms. We can say, with relative certainty, that surfaces of iron inside closed containers deteriorate or rust in a different way from surfaces completely in the open, and that deterioration is probably related to a chemical or microbial contaminant of the atmosphere usually present in the tropical forest.

In this same experiment, parallel exposures of surfaces of the metals aluminum, silver, and chromium were made. Results with silver were similar to those reported for iron. Aluminum and chromium were only slightly affected by the short, one week exposure to the various environmental conditions described.

To interpret these results adequately, we need to know more than we presently do about the microbial and chemical content of tropical atmospheres. However, whether microorganisms are important in metals deterioration or not, we know beyond question that they do play a part in the deterioration of other kinds of materials. To that end, studies to increase our level of knowledge in these areas have been started.

With respect to further microbiological observations, Table 1 will illustrate how airborne microorganisms vary greatly from season to season, from time to time within the day, and from level to level in the forest environment. Note that there are many more microorganisms present in the dry season than in the rainy season; below treetop level than above the trees. Also, numbers of microorganisms appear to vary with the time of day. We will need much more data than we presently have before we can even begin to explain these large differences in the numbers of airborne microorganisms we encounter in the forest environment.

We have begun to make periodic observations of surface microbiology in the tropical environment and to examine what we believe is a closely related element of the environment; namely, atmospheric chemistry. By way of illustration, I'd like to show you three results that may prove to be significant. One has to do entirely with microbiology. One shows why we think that there may be a definite

relationship between surface microbiology and atmospheric chemistry; and one is a very preliminary report on some chemical analyses of air samples.

1. We checked the leaves of twenty of the more common forms of vegetation in a semideciduous forest area. Thirty-six separate groups of fungi were identified.* Of the thirty-six types, twelve were present in both the dry and rainy seasons, twelve were present only in the rainy season, and twelve were present only in the dry season. This seems, at first glance, to be a normal and possibly expected distribution. However, when we look specifically for members of the groups inhabiting specific plants, we find some rather surprising things. The numbers in Table II illustrate this. Numbers of identified types inhabiting leaves of individual plants during the rainy season never exceeded four. The average number of types of fungi per plant was 2.3. On the other hand, the number of types of fungi on individual plants in the dry season ranged between three and twelve, the average number being 9.5. Perhaps the most interesting observation of all was the almost complete change of the fungi which could be cultivated from individual leaves from rainy to dry season. The third column of the Table notes the incidence and frequency of overlaps of types for each plant. Note that for eight of the twenty kinds of vegetation there was no overlap from rainy to dry season. Ten plants maintained one type of fungus in both seasons. Only two plants maintained two types of fungi in both seasons. None of the plants maintained any more than two types of fungi for both seasons. Overlapping involved only four of the thirty-six forms of fungi. *Hormodendrum* was the persistent fungus in six instances; *Phoma* in four; *Fusarium* persisted only once. *Aspergillus* appeared in both cases of double overlapping; one time each with *Hormodendrum* and *Phoma*.

There were a few forms of fungi present in the rainy season which could not be identified. This was mainly because they could not be brought to the fruiting stage necessary to permit identification. There were at least forty additional types, not identifiable, present in the dry season. This exaggerates even more the indicated 4 to 1, dry to rainy season ratio of numbers of fungi residing on leaves, and may tend to explain the large numbers of organisms present in air during the dry season.

2. We have evidence that some substance in the atmosphere as vapor may be related to growth of microorganisms. For example, Plate 12 is a picture of a non-nutrient agar gel surface on which microorganisms would grow if nutrient were present. There are no

* Identification was provided, for the most part, by two consulting mycologists: Prof. Eugene Staffeldt, New Mexico State University, Las Cruces, New Mexico; and Mr. Oscar Calderon, White Sands Missile Range, White Sands, New Mexico.

microorganisms here because we have taken great care to remove all nutrient from the agar and have not inoculated it with any organisms. A gel surface inoculated with fungi typical of a forest fungus community* produced only a barely visible growth of microorganisms, indicating that we were not transferring nutrient with the inoculum. Plate 13, similarly inoculated, has added to it the volatile chemicals that we were able to condense out of two thousand liters of air using a glass surface condenser cooled to liquid nitrogen temperature. Note that a modest amount of microbial growth occurred. A non-nutrient agar gel surface containing condensate and the spores and mycilial debris that may have been collected with the condensate did not produce very much microbial growth (not shown).

This indicates either that only certain microorganisms of the community grow on the cold trap condensate or that growth may be dependent upon some synergistic effect requiring the presence of the whole community.

Not all cold trap samples will support the growth of fungi, and the value, if any, of this demonstration of the relationship between the tropical microbial community and the chemistry of the atmosphere is yet to be determined. No doubt, the really definitive findings concerning the chemistry of the atmosphere will be obtained by more conventional methods.

3. Our efforts to undertake a study of the chemical content of the tropical atmosphere have been materially furthered through a cooperative arrangement with members of the staff of the National Center for Atmospheric Research at Boulder, Colorado. The cooperative nature of this work is unique. Basically, the work will be done to determine levels of trace gases in the atmosphere at various sites and regions. The data will be used by the National Center staff to extend already existent studies such as those published by Junge (1963), in which the objective is to explain the behavior of the world air mass in terms of interaction of air with water, atmospheric pollution by human activity, the influence of sunlight and rain, and the overall influence of vegetation. Data will be used at Tropic Test Center to interpret, on a comparatively micro-scale, the effects of concentrations of chemicals in the atmosphere on microbial growth, materials deterioration, and the like. Complete results of chemical studies will be reported elsewhere. Preliminary observations on analyses of atmospheric gases indicate that, contrary to expectations, the concentrations of reduced organic volatile organic compounds, except for high levels of ammonia, the primary decomposition products of decomposing vegetation found in the forest environment, are surprisingly low. Hydrogen sulfide, one of the substances most expected, was not detected at all by the methods used. Aldehydes, a possible product of

* <u>Hormodendrum</u>, <u>Chaetomium</u>, <u>Fusarium</u>, <u>Stemphylium</u>, <u>Mucor</u>, <u>Pyrenochaeta</u>.

living vegetation, were found in higher than expected concentrations. The oxides of nitrogen and sulfur were present in concentrations which Junge would consider normal to slightly above normal.

Summary: The practical nature of the studies undertaken is emphasized by reference to the known tendency of the tropical environment to accelerate decomposition of organic materials. Several experiments conducted in a tropical environment offer strong, though not conclusive, evidence that microbiological activity deteriorates metals. Data were presented to show that the presence of microorganisms, and/or products of microorganisms, water, and cycling of temperature to force frequent wetting of surfaces, produced an effect on iron different from the effect caused by exposure to the ambient atmosphere of the Canal Zone. Furthermore, it was shown that unless these conditions were all present--microorganisms, water, and temperature fluctuations--iron surfaces remained unaffected. Supplementary data were presented to show that microorganisms may derive at least some nutrient from atmospheric chemical contaminants, and very preliminary statements were made concerning the results of atmospheric sampling done to determine the nature of the contaminants present in the tropical atmosphere.

References:

1. PDC Search No. 65-027-3, dated 11 May 1965.

2. PDC Search No. 63-025, dated 11 July 1963.

3. Lodge, J. P., and Frank, Evylyn. Air and Water Pollution, 6, pp. 215-221, 1962.

4. Junge, Christian E. Air Chemistry and Radioactivity, International Geophysics Series, 4, Academic Press, 1963.

TABLE I

The influence of season, elevation, and time of day on viable microorganisms/hr falling on a 100 cm^2 horizontally exposed surface.

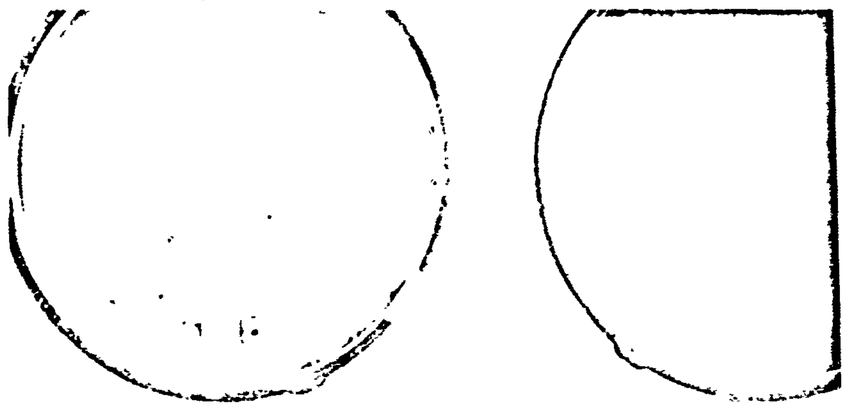
LOCATION ALBROOK FOREST		July 1965 Rainy Season						March 1966 Dry Season					
		Fungi			Bacteria			Fungi			Bacteria		
	Time of Day	High	Low	Av*	High	Low	Av*	High	Low	Av*	High	Low	Av*
5 feet above ground (under forest canopy)	1200-1300	177	23	102	34	9	25	3,025	27	950	2,920	6	610
	1600-1700	205	6	106	76	30	54	1,110	515	813	1,670	333	1000
	1900-2000	239	31	163	65	25	46	353	44	175	219	46	102
120 feet above ground (above forest canopy)	1200-1300	117	15	70	54	17	49	248	48	132	293	0	68
	1600-1700	151	7	103	60	22	45	38	30	35	43	24	33
	1900-2000	243	36	154	69	25	60	123	38	102	121	21	52

* Average of the 8-10 observations made at irregular intervals during indicated period.

TABLE II

Numbers of types of fungi on leaves of trees in rainy and dry seasons

Type of Vegetation	No. of Types of Fungi on Indicated Vegetation		No. of Overlaps - Rainy to Dry Season
	Rainy Season	Dry Season	
Serjania mexicana	4	8	0
Copaifera panamensis	1	6	0
Hirtella racemosa	2	9	0
Lycoseris latifolia	2	8	1
Triumfetta lappula	1	11	0
Adiantum lucidulum	2	10	1
Piper villiramulum	4	8	1
Amaioua corymbosa	3	8	0
Sparganophorus sp.	2	9	1
Anacardium excelsum	2	12	1
Machaerium sp.	4	6	2
Cordia alliodora	1	3	0
Calopogonium sp.	2	9	1
Panicum maximum	2	4	1
Lacistema aggregatum	4	9	2
Xylopia aromatica	2	9	1
Alibertia edulis	2	7	0
Lantana camara	1	11	1
Apeiba tibourbon	3	6	0
Gouania polygama	3	7	1
No. of fungus/plant combinations	47	191	Incidence of overlap: None: 8 times; One type: 10 times
Av. no. of fungi/plant	2.3	9.5	Two types: 2 times; More than two types: None



a. forest b. open
Plate 6 exposure in tube

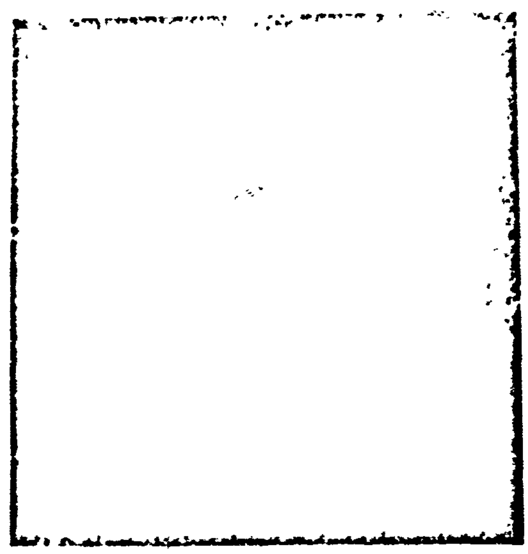
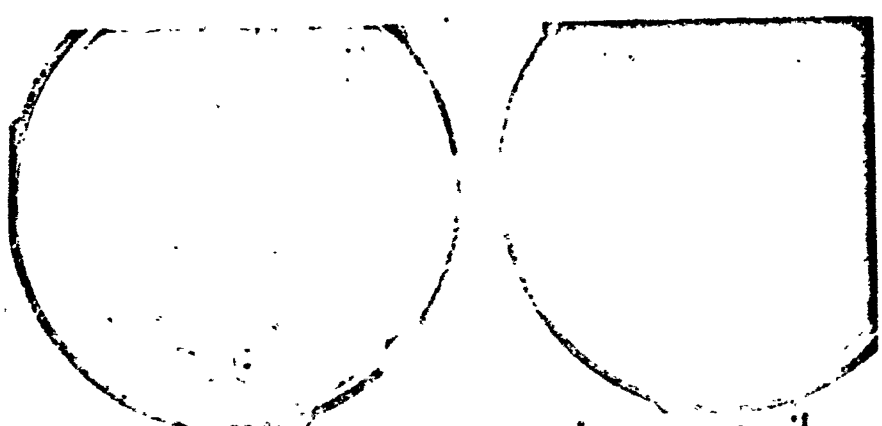


PLATE 7



a. tube b. on soil surface (covered)
Plate 8

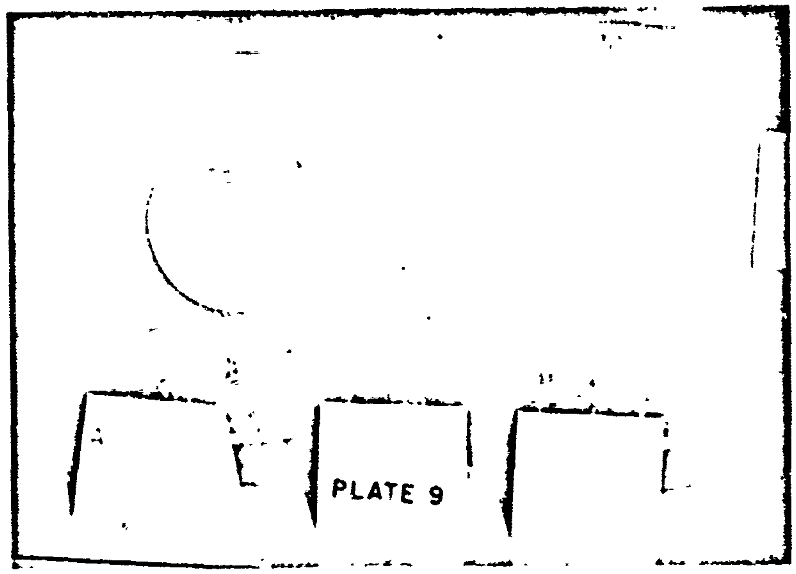


PLATE 9

PLATE 10

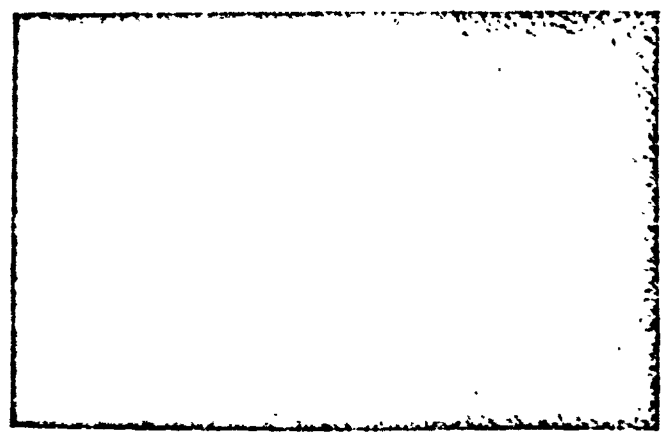


PLATE 11

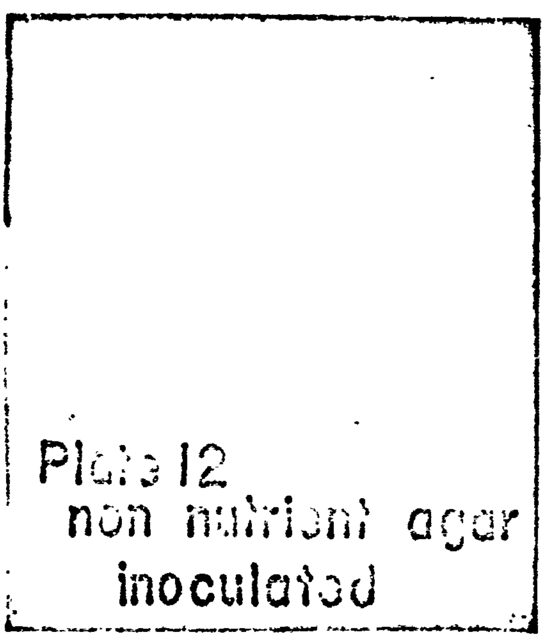


Plate 12
non nutrient agar
inoculated

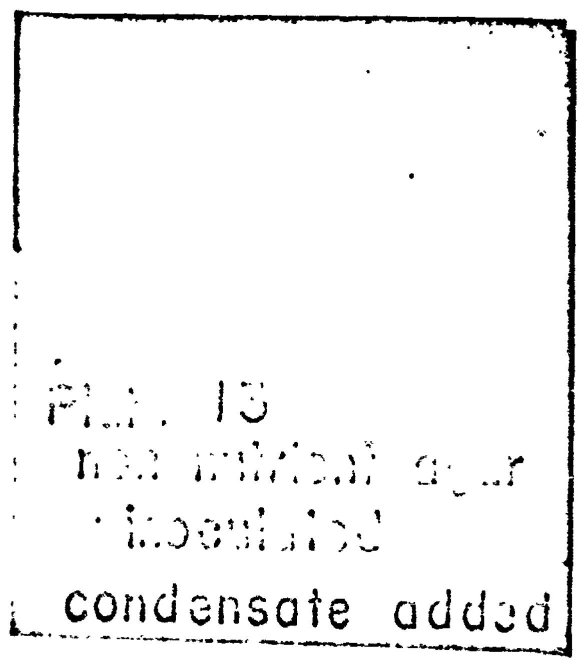


Plate 13
non nutrient agar
inoculated
condensate added